U. S. DEPARTMENT OF AGRICULTURE,
OFFICE OF EXPERIMENT STATIONS.

THE PHYSIOLOGICAL EFFECT OF CREATIN AND CREATININ

AND

THEIR VALUE AS NUTRIENTS.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Office of Experiment Stations,
Washington, D. C., April 19, 1899.

Sir: I have the honor to transmit herewith a report on the physiological action of creatin and creatinin, and their value as nutrients, prepared by Prof. J. W. Mallet, of the University of Virginia, under the supervision of Prof. W. O. Atwater, special agent in charge of nutrition investigations, in accordance with instructions given by the Director of this Office.

The nitrogenous extractives which occur in animal and vegetable foods are bodies whose value in nutrition is a matter on which definite information is lacking. It has been commonly assumed that they supply the body with energy, and hence serve to protect protein, although they can not serve to build tissue. Creatin is one of the principal constituents of most meat extracts, and since such preparations are commonly used as foods or in the preparation of foods, definite knowledge of the true food value of creatin is a matter of practical importance. Professor Mallet was especially fortunate in securing large quantities of creatin and creatinin for use in the experiments, and owing to the abundance of material was able, in addition to physiological investigations, to make many comparative tests of the chemical properties of these bodies, some work of this nature being necessary, since the statements found in text-books concerning the properties of creatin and creatinin and the chemical methods of separating them are not in harmony.

The report is transmitted with the recommendation that it be published as Bulletin 66 of this Office.

Respectfully,

A. C. True,
Director.

Hon. James Wilson,
Secretary of Agriculture.

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THE PHYSIOLOGICAL EFFECT OF CREATIN AND CREATININ.

INTRODUCTION.

The nitrogenous extractives, creatin and creatinin, are bodies the physiological importance of which should be determined. Creatin is a normal constituent of flesh, forming about 0.3 per cent of the total nitrogenous material of muscular tissue. It is one of the principal constituents of most meat extracts. The total amount of creatin in the human body may be taken to be something like 90 gm. Creatinin is a normal constituent of human urine. Opinions vary concerning the food value of creatin, creatinin, and other nonproteid amidic bodies. The opinion has often been advanced that such bodies may serve to protect protein, although they can not serve to build tissue. If they protect protein, it would seem reasonable to suppose that they must yield energy in the body. The general question as to how far the nonproteid amidic substances, which occur most abundantly in foods of animal or vegetable origin, or as the first products of metabolism, are capable of undergoing oxidation in the human body, and thus contributing to the production of energy, is a subject on which more information seemed desirable. The usual methods of determining such bodies are not altogether satisfactory. Therefore, the investigations reported in this bulletin were made, some studies of the chemical properties of flesh bases and the methods of separating them being necessary before the physiological work could be undertaken.

It has been found necessary to restrict the scope of the inquiry to the so-called flesh bases, creatin and creatinin. An abundant supply of asparagin and a considerable quantity of one or two other amidic compounds of vegetable origin have been procured, but these have not yet been worked with. In the case of asparagin, more has already been done by others in the line of experimenting proposed and less interest attaches to its relations to metabolism in the human body.

Such relations in the case of creatin and creatinin possess a double interest. On the one hand, creatin is a constituent of flesh food, and it is desirable to know whether and to what extent it becomes the material for metabolic change and thus contributes to the production of

energy. On the other hand, the same substance is formed from proteid metabolism in our own muscles and to a much smaller extent in nervous and other tissues, and it becomes a question of importance what becomes of this creatin, and in what form is it eliminated. There is no reason to believe that creatin or creatinin can be utilized as synthetic material for the building up of the proteid tissues of the body. Creatin is easily changed into its anhydrid, creatinin, and this latter substance is found habitually in the urine, so that it might be supposed to represent the slightly changed form in which both the creatin in food and that originating in the living muscles are eliminated. But the quantity of creatinin given off daily in the urine is in general quite small and, although sufficient as a rule to account for the creatin of the flesh food consumed, the amount seems hardly large enough to cover also the creatin which may be supposed to originate in the living organism in the same time. Hence it has been suggested as probable that in some part of the body creatin may become converted into other and more complete cleavage products of metabolism, being in large part at least ultimately eliminated as urea. Should we, therefore, consider creatin and creatinin as representing nitrogenous material—received into the living body from without or formed in the body itself—which escapes further metabolism (save the mere conversion of one of these two closely related compounds into the other) and is simply excreted without contributing to the production of energy, or as representing an intermediate stage in the metabolism of protein which on further cleavage does in fact contribute something to the general output of energy of the body.

In connection with this latter hypothesis it may be noted that Stohmann and Langbein[1] found the molecular heat of combustion of creatin (anhydrous) to be 560 calories,[2] and that of urea to be 152.2 calories per gram molecule. Supposing all the nitrogen of creatin to assume the form of urea, and neglecting any thermic result of the formation or oxidation of by-products, as two molecules of creatin will yield three molecules of urea, 331.1 calories will represent the heat evolved for each gram molecule of the former converted into and eliminated as the latter substance.

VIEWS OF PHYSIOLOGISTS REGARDING THE RÔLE OF CREATIN AND CREATININ.

The divergence and uncertainty of the views held on this subject by leading physiologists are indicated by the following condensed extracts from the works of well-known authorities.

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[1] Quoted in Beilstein's Handbuch der organischen Chemie, 3d ed., Vol. I, p. 1189. See also article by Stohmann on "The heat equivalent of the nutrients of food," Experiment Station Record 6, p. 598.

[2] The results of new experiments which Professor Atwater has kindly made with his improved bomb calorimeter on the heat of combustion of both creatin and creatinin furnished by the writer are given on p. 13.

Thus, Martin[1] says:

In seeking antecedents of urea one naturally turns first to the muscles, which form by far the largest mass of proteid tissues in the Body. Analysis shows that they always contain creatin, a body intermediate chemically between proteids and urea. The quantity of this in muscles is practically unaffected by work, and is from 0.2 to 0.4 per cent. Since it is readily soluble and dialyzable and, therefore, fitted to pass rapidly out of the muscles into the blood stream, it is a fair conclusion that a good deal of it is formed in the muscles daily and carried off from them. Creatin, too, exists in the brain, and probably there and elsewhere in the nervous system is produced by chemical degradation of protoplasm; the spleen also contains a good deal of creatin, and so do many glands. This substance would therefore seem to be constantly produced in considerable quantities by the protoplasmic tissues generally; and since it belongs to a group of nitrogenous compounds which the Body is unable to utilize for reconstruction into proteids it must be carried off. The urine, however, contains very little creatin or its immediate derivative, creatinin, and what it does contain depends mainly on the feeding, since it varies with the diet and vanishes during starvation; so it is probable that this substance is converted into urea and excreted in that form. * * * While the urea resulting from further changes in the creatin formed in the tissues is a measure of the wear and tear of their protoplasm, part of the urea excreted probably has a different source; being due to the oxidation of proteids, as energy liberators or respiratory foods, before they have ever formed a tissue. When plenty of proteid food is taken the urea excretion is largely increased and that very rapidly, within a couple of hours for example, and before we can well suppose the proteids consumed to have been built up into tissues, and these in turn broken down; in fact, there need be, and usually is, under such circumstances, no sign of any special activity of any group of tissues such as one would expect to see if the urea always came from the breaking down of formed histological elements. * * * In artificial pancreatic digestions, when long carried on, two bodies, called leucin and tyrosin, are produced from proteids. It is found that when leucin is given to an animal in its food it reappears in the urine as urea; so the Body can convert leucin into that substance. Hence, a possible source of some of the *luxus-consumption* urea is leucin produced during intestinal digestion; and this is very likely turned into urea in the liver. At any rate the liver, to which the portal vein might carry all leucin thus formed, contains urea, which no other gland does; and when the liver is greatly altered, as in phosphorus poisoning and the disease known as acute yellow atrophy, urea almost entirely disappears from the urine. This latter fact seems to point to a final production of urea in the liver, whatever its immediate antecedents may be; whether muscle creatin, or intestinal leucin, or excess of peptones in the diet.

Landois[2] summarizes the subject as follows:

Although it is surmised that some of the nitrogenous bodies named above, more especially leucin, and perhaps also creatin, are the precursors of urea, yet we can not say definitely how or where the transformation takes place. Perhaps this is effected in the liver, and, it may be, also in the spleen.

The evidence in support of the theory that the liver is the seat of urea production is discussed further on.

Von Noorden's[3] opinions are in effect as follows:

Creatin is undoubtedly a derivative of albumen. It is widely distributed in the body, principally in the muscular system, in which it is contained up to 0.3 per cent (O. Nasse). Creatin rarely comes under consideration as a final product of

[1] H. Newell Martin; The Human Body, 1881, pp. 433–435.

[2] L. Landois; A Text-book of Human Physiology, 1891, p. 505.

[3] Carl von Noorden; Lehrbuch der Pathologie des Stoffwechsels, 1893, pp. 70, 71.

metabolism, since before its elimination it passes, probably in the kidneys, into its anhydrid, creatinin. Only in case of alkaline reaction of the urine can a reverse change, into creatin, occur, in the bladder, or after discharge of the urine.

The greatest part of the creatinin in the urine comes from the food, since the creatin of the food, easily absorbable from the alimentary canal, passes through the body unchanged, without being oxidized and is, all of it, discharged as creatinin. He who eats much meat discharges much creatinin; hence it is found more abundantly in the urine of men, adults, and persons in health than in that of women, children, and sick persons; on the other hand it either occurs in very small quantity or is altogether wanting in the urine of children fed only on milk (free from creatin). The average quantity discharged per day is about 1 gram for an adult, healthy, and fully fed man; for a woman, 0.65 gram.

A second portion of the creatinin of the urine comes from creatin which is formed in the body itself, probably in the muscle cells. Only this part is to be regarded as a product of the metabolism of albumen. The muscles contain, as has been mentioned, creatin in abundance; in the urine, apart from the creatin of the food, it occurs only in traces. Whether these traces represent the whole of the creatin metabolism in the muscles, whether in reality the quantity of creatin, as a whole, remains constant and only these traces are worn off, is unknown. If the change in the stock on hand be a greater one it would have to be supposed that creatin is in the muscle itself converted into urea or other nitrogenous compounds, since leaving the muscle cell as creatin it could only appear unchanged in the urine. * * * Under conditions in which the muscular system of the body is rapidly consumed much creatin is eliminated. (See sections on Starvation and Fever.)

In a very recent treatise Howell[1] says:

What becomes of the relatively large quantity of creatin in the muscles? It has been suggested that it is one of the precursors of urea; that it represents an end product of the proteid destroyed in muscle which is subsequently converted to urea in the liver or elsewhere. This supposition is supported by the fact that creatin may be decomposed readily in the laboratory, with the formation of urea among other products. But against this theory we have the important fact that creatin introduced into the blood is not converted into urea, but is eliminated as creatinin.

The subject is discussed at length by Foster:[2]

Urea does not arise in muscular substance itself as one of the immediate waste products of muscular substance.

There is, however, always present in relatively considerable amount, on an average about 0.25 per cent of wet muscle, a remarkable body, *creatin*. This is in one sense a compound of urea: it may be split up into urea and sarcosin. This latter body is a methyl glycin; that is to say, a glycin in which methyl has been substituted for hydrogen, and glycin itself is amido-acetic acid, a compound of amidogen; that is, a representative of ammonia and acetic acid. Hence creatin contains urea, which has close relations with ammonia, together with another representative of ammonia, and a surplus of carbon and hydrogen arranged as a body belonging to the fatty acid series. * * * In dealing with the chemistry of muscle we saw that urea was conspicuous by its absence from the extract of muscle, whereas a very appreciable quantity of creatin was invariably present, and indeed was the prominent nitrogenous crystalline constituent of that extract. It seems difficult to resist the conclusion that creatin is the main normal nitrogenous product of the metabolism of skeletal muscles. If we accept this view, then upon the fact of the presence

[1] William H. Howell; An American Text-book of Physiology (chapter on Digestion and Nutrition), 1896, p. 279.

[2] M. Foster; A Text-book of Physiology, 1895, pp. 93, 590–595.

of creatin in, and the absence of urea from, the muscle itself, we may base the conclusion that while the muscle produces creatin as an antecedent of urea, the creatin so produced is converted into urea in some part of the body other than the muscle itself. * * * We must not, however, leave this statement without referring to a difficulty. Creatinin, as we have seen, is so frequently found in urine as to be regarded as a normal constituent, at all events of human urine; and creatinin is, as we have seen, the urinary form, so to speak, of creatin; the one body easily changes into the other by the assumption or removal of H_2O. This suggests the question: Is not the creatinin of urine the representative of the creatin of the muscles, which is thus excreted directly without undergoing the change into urea just discussed? In answer to this we may say in the first place that the quantity of creatinin in urine, though variable, is small; we may put the average at about 1 gram in 24 hours. Now, muscle contains from 0.2 to 0.4 per cent of creatin; and this, taking the total muscle of the body (to say nothing of other sources of creatin which we shall mention presently) at about 30 kilos, would give 60 to 120 grams creatin as present in the muscles of the body at any one moment. We can hardly suppose that the metabolism of muscle is so slow as out of this stock only to provide the 1 gram of creatinin in twenty-four hours. Moreover, the creatinin in urine vanishes during starvation, is very markedly increased by a diet of flesh which contains creatin, and is not increased either by muscular exercise (which, however, would only indirectly affect the nitrogenous metabolism of muscle) or by such conditions—fever, for instance—as notably increase the urea of urine by increasing the nitrogenous metabolism of muscle. We infer, therefore, that the normal presence of creatinin in urine is due to the direct administration of creatin present in a (normal) flesh diet, and has nothing to do with the muscular metabolism of the individual who is secreting the creatinin in his urine.

The fact, however, that the creatin present in the muscle of the food and absorbed from the alimentary canal does not undergo a change into urea, but is excreted as creatinin—that is, virtually as creatin—warns us to be careful in adopting the conclusion arrived at above, that the creatin produced by muscular metabolism in the living body is a conspicuous antecedent of the urea of the urine. It is difficult to see why creatin passing into the blood of the capillaries of the muscle should be changed into urea, while that which passes into the capillaries of the portal system is not; for reasons which will be apparent presently we should rather expect that the latter, being more directly exposed to the influence of the liver, would be more readily and more completely converted than the former. Indeed, the question forces itself upon us: Is creatin, after all, the natural main product of the nitrogenous metabolism of muscle? Is it possible that in the normal metabolism of the living muscle the nitrogen leaves the muscular substance and passes into the blood in another form, as some substance not creatin, and that it is as the muscle dies that creatin is formed, just as the solid myosin is unknown to the living fiber, but makes its appearance in a dying one? We have no positive evidence, however, that this is so, and meanwhile may continue to suppose that creatin is formed, and that in consequence creatin is a conspicuous antecedent of the urea of the urine; but we must not regard this as proved * * * we may probably consider the metabolism of the nervous system as a mere addition to that of the muscular system, at least as regards the point on which we are now dwelling. The amount of nitrogenous metabolism taking place in connective tissue, cartilage, bone, and the skin is probably still less. * * * The nitrogenous metabolism of the glands, however, more particularly that of the liver, does deserve special consideration * * * we have seen that pancreatic juice may carry part of the proteids on which it acts beyond the stage of albumose and peptone, and reduce that part into leucin, tyrosin, and other bodies. * * * We have seen reason to think that proteids of a meal are absorbed, not by the lacteals, but by the portal blood vessels, and such bodies as leucin probably take the same course. This being so, all these bodies pass through the liver and are subjected to such influences as may be exerted by the hepatic

cells * * * we have, however, a convergence of evidence that the last stage of the process, namely the conversion into urea of some or other product of proteid metabolism which although allied to is not exactly urea, does occur in the liver. In the first place, a large quantity of urea seems to be present in the liver of mammals; in this respect the liver presents a strong contrast to the muscles. * * * In the second place, in certain cases of a form of disease of the liver known as acute yellow atrophy, in which the hepatic cells are so changed that their functional activity is largely diminished, the urea of the urine not only undergoes a very marked decrease, but appears to be replaced to a very large extent by leucin. This fact suggests that leucin (and not, for instance, creatin) is the chief immediate product of the nitrogenous metabolism of the body, and that the leucin thus produced is, in a normal state of things, converted into urea by the liver.

PREVIOUS RESEARCHES UPON THE NUTRITIVE VALUE OF CREATIN AND CREATININ.

There is but a small amount of direct experimental evidence as yet recorded bearing on the fate of creatin or creatinin introduced as such into the animal organism.

Ph. Munk[1] determined urea and creatinin in the urine of dogs when 2 grams of creatin was injected into a vein, and under normal conditions. He concluded that the injection of the creatin increased (certainly) the amount of creatinin and (doubtfully) the amount of urea excreted. He did not determine whether any unaltered creatin was excreted. He also experimented to a very limited extent upon himself, taking by the mouth a rather small quantity of creatin, and reached similar results. Thus on a mixed diet he found in the urine when no creatin was taken 16 to 20.5 grams urea and 0.77 to 1.23 grams creatinin; when 5.5 grams creatin was taken, 21.8 grams urea and 1.48 grams creatinin. The amount of urea reported seems remarkably low, assuming it to represent the excretion of twenty-four hours, and the increase in the amount of both urea and creatinin excreted is not great, while the creatinin represents but a small part of the creatin taken.

Meissner[2] also injected solutions of creatin and creatinin into the blood of dogs and rabbits. He found that creatin was eliminated with great rapidity by the kidneys. Creatinin at first passed unchanged into the urine but afterwards seemed to undergo decomposition, no more creatin or creatinin being found. In the case of the dogs with a meat diet he found creatin as well as creatinin always present in the urine. Voit criticised these experiments in which the substances were injected into the blood on the ground that *time* enough for metabolism is not given. In support of this objection he cited the fact that a large amount of sugar can be fully disposed of when it has been swallowed, while a few grams injected into the blood will appear unchanged in the urine.

Voit[3] made some experiments with dogs, taking into account the quantity of creatin contained in the meat with which they were fed,

[1] Deut. Klinik, 1862, p. 299.

[2] Ztschr. rat. Med., 3. ser., 24 (1865), p. 100; 26 (1866), p. 225.

[3] Ztschr. Biol., 4 (1868), p. 77.

and aimed at showing that, as more creatin (in the form of creatinin) was recovered from the urine than was given in the meat food, the surplus must have been formed in the living body. He came to the conclusion that in the breaking up of proteids in the muscles a certain part of the nitrogen takes the form of creatin, and is given off as such (or, rather, for the most part changed to creatinin) in the urine. He deemed it very improbable that creatin is changed into urea, the amount of creatin in the urine rising or falling in proportion to the amount taken in in flesh food plus the amount due to proteid metabolism in the muscles.

Voit also made some experiments on a dog, adding creatin and creatinin in separate form to the food. He concluded that creatin and creatinin are not changed to urea, of which there was no sensible increase, but that the greater part is removed in the urine, the failure to recover all being perhaps due to a part being held back in the alimentary canal in consequence of sparing solubility. He also reported that he found under the administration of creatinin the urine may become temporarily alkaline, creatin being then given off, formed from creatinin in the body of the animal experimented on.

It may be added that Max Rubner[1] found by direct experiment on a dog with a respiration apparatus that the flesh bases of meat extract have no influence on the elimination of carbon dioxid or the production of heat.

It seemed, therefore, that experiments were desirable which might throw further light on this debatable question of the relations of creatin and creatinin to the chemistry of animal life.

MATERIALS EMPLOYED IN THE PRESENT INVESTIGATION.

A good deal was hoped from the use in experiment of larger quantities of the flesh bases in a state of purity than had previously been used, so that from the methods (necessarily not rigorously exact) of determining these substances as eliminated in the urine, results of a fairly decisive character might be obtained. The writer was fortunate enough (thanks to the kindness of a friend and former pupil, now connected with a firm preparing a well-known meat extract) to possess several hundred grams each of almost chemically pure creatin and creatinin. These were very carefully purified further and recrystallized, and furnished abundant material for the physiological experiments undertaken. They were quite free from mineral impurity of any kind. Each was tested for the presence of the other, and, by the fractional use of solvents, each was ascertained to be quite uniform in character.

The general properties of the creatin and creatinin so prepared were found to agree in general with the description given in the text-books, but a few special points of detail may be noted.

According to Liebig's description creatin "has a somewhat bitter taste and scratches in the throat," while "creatinin in the state of concentrated solution has a caustic taste like that of dilute ammonia."

[1] Ztschr. Biol., 20, p. 265.

Both of these substances as prepared by the author when taken into the mouth in large quantity and swallowed with a little pure water, had a bitter taste, not very strong or disagreeable. This taste was comparatively slight in the case of creatin and much more marked in that of creatinin, as might be expected from their different degrees of solubility.

The statements of Liebig and G. S. Johnson do not agree in regard to the solubility of creatinin in alcohol, the former finding it three times as soluble as did the latter. My determinations gave the following results: One hundred grams of 90 per cent ethyl alcohol dissolved 0.018 gram of creatin or 0.454 gram of creatinin at 19.2° C. Under these conditions, therefore, creatinin is more than twenty-five times as soluble as creatin. For the analytical separations to be made it was important to know also the solubility of urea in alcohol of the strength employed. On the authority of Prout it is stated that one part of urea dissolves in five parts of alcohol of specific gravity 0.816 in the cold. I found that 100 grams of 90 per cent alcohol dissolved 11.526 grams of pure urea at 19.2° C., so that under these conditions it is more than twenty-four times as soluble as creatinin.

Some discrepancy has also been observed in the recorded statements as to the reactions of these flesh bases with litmus paper. According to the original observation of Chevreul, uncontradicted by later experimenters, creatin is neutral to vegetable colors, while Liebig and others have reported creatinin as distinctly alkaline, changing red litmus to blue and reddening turmeric. However, Salkowski found that a strongly alkaline sample left an alkaline ash on ignition and that pure creatinin was quite free from alkaline reaction. The creatin used by me was quite neutral to litmus paper; the creatinin, although free from ash, gave an extremely faint, scarcely perceptible, alkaline reaction.

In view of the readiness with which creatin may be converted into creatinin, and vice versa, and in view also of the fact that for the purposes of this investigation they would have to be sought for and determined in the presence of the urea of urine, the behavior of creatin, creatinin, and urea with reagents had to be examined. In this direction, also, results were obtained not altogether in accord with the statements of some of the text-books in general use. Thus, Allen[1] says: "Creatin is also distinguished from creatinin by being unprecipitated by a solution of phosphotungstic acid in presence of hydrochloric acid."

Using fairly strong aqueous solutions of the flesh bases, I found that the hydrochloric acid solution of phosphotungstic acid gave with creatin a small crystalline precipitate, forming gradually; with creatinin also a precipitate, but more copious, and forming more promptly.

In O. Hammarsten's Text-book of Physiological Chemistry[2] the statement is made in reference to creatinin that "it is precipitated like urea, with mercuric-nitrate solution." I found that even the most concen-

[1] A. H. Allen; Commercial Organic Analysis, vol. 3, Pt. III, p. 287.
[2] Authorized translation, by J. A. Mandel, p. 348.

trated aqueous solution of creatin or creatinin gave no precipitate with a solution of mercuric nitrate containing just enough free nitric acid to make it clear, while the reagent gave an abundant precipitate with a solution of urea. If to the mixture of creatin or creatinin solution with the acid mercuric nitrate a dilute alkali was added, a white precipitate was readily formed.

It may also be mentioned that the specially valuable precipitant for creatinin, zinc chlorid,[1] also gives precipitates—flocculent rather than crystalline—with both creatin and urea. These precipitates are moderate in amount, but gradually increase, so that separation from these substances must be effected before the precipitation of creatinin-zinc chlorid can be made the basis for an accurate determination.

It seemed desirable to secure new determinations of the heat of combustion of creatin and creatinin, using a portion of the material prepared for these experiments. Such determinations were made, by the kindly offered aid of Prof. W. O. Atwater, in the chemical laboratory of the Storrs Connecticut Experiment Station, at Middletown, Conn., with the improved form of bomb calorimeter. The specimens of air-dried creatin and creatinin were dried in air for 5 hours at 96°, and gave for water-free substance, creatin 88.08 per cent and 88.075 per cent, and creatinin 93.66 per cent and 93.70 per cent.

These dried samples were burned, and yielded results as below. They kindled with great difficulty, not even naphthalene serving to ignite them. The substance was finally burned by inclosing in a gelatin capsule of known weight and making allowance for the heat of combustion of the capsule. The heats of combustion of 1 gram of water-free substance were, in large calories:

	Calories.
Creatin, first combustion	4.269
Creatin, second combustion	4.264
Average	4.267
Creatinin, first combustion	4.578
Creatinin, second combustion	4.588
Average	4.583

From these results we have, as the value for the molecular heat of combustion: Creatin, 559 calories; creatinin, 517.9 calories; and the conversion in the human body of each molecule (counted in grams) of creatin into creatinin involves heat *consumption* to the extent of 41.1 calories.

METHOD DEVISED AND EMPLOYED FOR SEPARATION OF CREATIN, CREATININ, AND UREA IN URINE.

Before stating the method worked out and adopted for the quantitative determination of the three substances, creatin, creatinin, and urea, in urine containing the usual normal constituents, it may be well

[1] The investigations of the author confirm the statement of Schäfer (Physiology, p. 100) that unless the proper conditions be exactly observed the precipitation of creatinin with zinc chlorid is very uncertain and unsatisfactory.

to note the character and strength of the reagent solutions used. It was desirable to have these in a fairly concentrated form, so as to reduce as much as possible the volume of liquid to be evaporated, and thus diminish the chance of creatin becoming converted into creatinin, or the reverse.

The reagents were as follows:

(1) Milk of lime, a thin fluid pulp of pure calcium hydroxid, for the most part in suspension, in water.

(2) Aqueous solution of pure neutral calcium nitrate (200 grams to the liter). The object of using nitrate rather than chlorid for the removal of phosphates, etc., was to avoid the addition of large quantities of mercuric nitrate, which would otherwise have been necessary before urea could be precipitated. A calcium rather than a barium salt was used, notwithstanding the consequent failure properly to remove sulphates. The calcium nitrate, added as such, as well as that subsequently formed in the liquid under treatment, is easily soluble in alcohol, while barium nitrate is not.

(3) Dilute nitric acid; 100 grams acid per liter of water.

(4) Aqueous solution of mercuric nitrate; 300 grams to the liter, with just enough free nitric acid to render the solution clear and prevent formation of a basic salt.

(5) Alcoholic solution of zinc chlorid; a nearly saturated solution in 90 per cent alcohol.

(6) Alcoholic solution of sodium acetate; a saturated solution in 90 per cent alcohol.

(7) Acetic acid; about 40 per cent.

(8) Sodium hypobromite, prepared by mixing equal volumes of an aqueous solution of sodium hydroxid (340 grams to the liter) and one of bromin (200 grams) and potassium bromid (240 grams) per liter.

To the portion of urine to be treated—usually about 250 or 300 cubic centimeters—there was added, with constant stirring, milk of lime until the reaction was distinctly alkaline and 20 cubic centimeters of the solution of calcium nitrate. After standing for an hour the turbid liquid was filtered, drained with the aid of a filter pump, and washed once or twice with a small amount of water. The filtrate was tested with a drop or two of the calcium nitrate solution in order to make sure that precipitation of phosphates, etc., was complete. It was then accurately neutralized with a few drops of dilute nitric acid, and urea was precipitated with the solution of mercuric nitrate, avoiding any large excess of the reagent. As soon as the flocculent precipitate had settled, and before it had time to become crystalline and granular, it was filtered off with a filter pump and washed sparingly with water. The filtrate was tested to make sure that the precipitation of urea was complete.

This precipitate was washed off from the filter into a beaker (owing to its bulkiness more than one filter had sometimes to be used), well stirred up with water, and decomposed by a rapid stream of sulphu-

reted hydrogen gas. The gas was passed through the liquid long enough to insure complete conversion of all the mercury into sulphid. The mercuric sulphid was removed by filtering, the filtrate evaporated to dryness at a gentle heat over a water bath, and the residual urea nitrate weighed, a check upon its amount being obtained by redissolving it, and from an aliquot part determining the nitrogen evolved by means of sodium hypobromite, adding pure potassium cyanate and introducing the bromin solution separately after the caustic soda, as recommended by Allen. This precipitation of urea by mercuric nitrate in acid solution is not complete, but it largely reduces the amount to be separated from the flesh bases. In some experiments urea was not determined and in some of these the precipitation with mercuric nitrate was omitted. In view of the observation of Meissner that the solubility of creatin in alcohol is increased by the presence of urea, it is better to remove the bulk of the urea in every case.

Through the liquid filtered off from the precipitate produced by mercuric nitrate a stream of sulphureted hydrogen was passed as long as it gave rise to any precipitate, and mercuric sulphid was removed by filtration. The filtrate was *very accurately* neutralized by milk of lime and evaporated at a temperature not exceeding 40° C. in a partial vacuum produced by a good filter pump. When the volume had been reduced to about 5 cubic centimeters, alcohol of 90 per cent was added in such quantity as would little more than suffice to hold in solution the quantity of creatinin supposed to be present. A little absolute alcohol was used to make, with the small quantity of water remaining unevaporated, a uniform strength for the whole of 90 per cent. The alcoholic liquid was allowed to stand for three or four hours, being occasionally stirred, and was then filtered.

The residue left upon the filter, having been lightly washed with 90 per cent alcohol, was treated with a small amount of boiling water and again filtered. The undissolved matter on the filter, consisting mainly of crystalline calcium sulphate, was washed twice with small quantities of boiling water. The aqueous filtrate was evaporated to dryness over a water bath, and the residue, creatin, was dried at 100° C. and weighed. The creatin was not absolutely pure, still retaining a little calcium sulphate and some organic compound of calcium which left lime on being strongly ignited. The creatin was redissolved and recrystallized, the microscopic appearance of the crystals was observed, and two or three tests for the identity of the substance applied (silver nitrate and potash, picric acid, etc.).

The alcoholic solution filtered off from the creatin, etc., was concentrated by evaporation to about one-fifth of its original volume; a little of the alcoholic solution of sodium acetate was added, with enough acetic acid to produce slightly acid reaction. The creatinin was then precipitated by the addition of alcoholic solution of zinc chlorid, allowing the liquid to stand in a well-covered vessel for three or four days before filtering. The creatinin-zinc chlorid was washed

on a dried filter with a moderate amount of 90 per cent alcohol, dried at 100° C., and weighed. The whole volume of alcohol, including the washings, was noted and a correction applied for the small amount of creatinin-zinc chlorid retained in it in solution. After weighing, the precipitate was carefully examined with a microscope to see that it was free from sodium chlorid or other visible impurities, and in two or three instances its identity was still further established by determinations of zinc and chlorin.

Having ascertained that the precipitation of creatinin by zinc chlorid was complete, a determination of such urea as remained in the filtrate was made with an aliquot part of it by means of sodium hypobromite and the result added to that from the urea previously recovered as nitrate.

In carrying out this process there was found to be considerable advantage in using of each reagent—e. g., mercuric nitrate and zinc chlorid—an amount calculated on the basis of approximate knowledge of the quantities present of the substances to be determined, such as to provide for a very small surplus only of the reagent, always, however, testing afterwards to make sure that enough had been employed.

Before proceeding to the physiological work a number of experiments were made to ascertain how far the analytical method might be trusted. Known amounts of creatin, creatinin, and urea were added to a portion of normal urine. Comparative determinations of these three substances were then made. Similar determinations were also made in another sample of the same urine which had not received such additions. Neglecting three or four preliminary experiments, in which the method was being worked out and in which the procedure was not uniform, the results given below were obtained. In each case the urine employed was the mixed total excretion of twenty-four hours. Of this two samples were taken, each one-fifth of the whole. To facilitate comparison with other results recorded later, the figures actually obtained have been multiplied by five, so that the report stands as for the whole daily quantity:

In the following table is shown the amount of urea, creatinin, and creatin found in nine samples of the total normal urine excreted in twenty-four hours.

Constituents of normal urine.

Number of sample.	Urea.	Creatinin.	Creatin.
	Grams.	*Gram.*	*Grams.*
1	29.246	0.781	.....
2	29.092	.832	.....
3	28.608	.894	.....
4	30.125	.695	.....
5	28.538	.753	.....
6	28.866	.844	.....
7	27.764	.869	.....
8	28.933	.815	.....
9	29.122	.872	.....

In the following table is shown the amount of urea, creatinin, and creatin added to ten samples of the same urine mentioned above, together with the amount and percentage of added material recovered.

Materials added to urine and amounts recovered.

Number of sample.	Additions made to urine.			Added materials recovered, *a*					
	Urea.	Creatinin.	Creatin.	Urea.	Creatinin.	Creatin.	Urea.	Creatinin.	Creatin.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*	*Per ct.*
1	2	1	.....	1.948	0.956	.....	97.4	95.6	.....
2	2	1	.....	1.941	.948	.....	97.0	94.8	.....
3	3	2	.....	2.895	1.898	.....	96.5	94.9	.....
4	5	3	1	4.846	2.856	0.863	96.9	95.2	86.3
5	5	4	1	4.721	3.903	.845	94.4	97.6	84.5
6	7	5	1	6.811	4.776	.856	97.3	95.5	85.6
7	8	5	2	7.736	4.635	1.769	96.7	92.7	88.4
8	8	8	2	7.592	7.609	1.774	94.9	95.1	88.7
9	10	10	3	9.683	9.392	2.635	96.8	93.9	87.8
Average	.....	.....	.....	.....	.....	.....	96.4	95.0	86.9

a After deducting from the amounts actually found those due to the normal constituents.

Although the process used is less favorable to the accurate determination of creatin than to that of creatinin, it may well be suspected that the difference between the two is not quite so great as seems to be shown by these figures, it being quite conceivable that, in spite of the precautions taken, some little conversion of creatin into creatinin may have occurred under the treatment applied. This would of course tend to raise the results obtained for the latter substance and to lower those of the former.

EXPERIMENTS UPON THE PHYSIOLOGICAL EFFECTS OF CREATIN AND CREATININ.

With a view to the experiments afterwards to be made on the ingestion of creatin and creatinin it was important to know what allowance should be made for the normal daily excretion of creatinin, and, moreover, as any increase in the elimination of urea in consequence of the ingestion of the flesh bases was one of the points to be examined, it was equally important to ascertain the normal daily excretion of urea. The figures of the first of the above tables afford a basis for the establishment of average values for the normal excretion of these two substances, and show about what range of variation from the average may be looked for. In order to make this range as small as possible, and consequently to give as much value as possible to the assumption of the average for purposes of comparison, care was taken during the whole of the time in which the physiological experiments were being carried on that the conditions of food, exercise, surrounding temperature, and sleep should be as uniform as was compatible with no very inconvenient disturbance of the ordinary habits of life. Especial attention was given to the selection of a simple mixed diet of animal and vegetable materials, not at all excessive, but fully sufficient in amount,

such as could be easily had with little change from day to day, for several weeks in succession. Without actually weighing the food consumed, a pretty close approach was made to uniformity in the quantity of each article, while the distribution in time of the meals was almost absolutely regular. The greatest variation probably was in the consumption of water in all forms, but this was of little importance, as all the samples of urine were weighed, not measured, and in all the experiments aliquot parts of the total daily excretion were employed. Judging by the values shown in the table (p. 16), the daily excretion of urea appears to have been 28.922 grams and that of creatinin 0.817 gram (as direct results of determination by the process used).

Having in mind the general belief in the well-marked effects of Liebig's and other meat extracts upon the nervous system, it seemed needful, when the physiological experiments were undertaken, to proceed with some caution in the administration of pure creatin and creatinin, and to begin with prudently small doses and gradually increase them. It was found, however, by a number of preliminary experiments, that 1 or 2 grams could be taken with no appreciable physiological effect; that 5 grams gave rise to noticeable symptoms, and that 10, or even 15 grams did not produce any very strongly marked or alarming impression. Not more than 15 grams was used in any of the experiments, since it was desirable that the quantity given should not be in excess of that which, even in the case of the less soluble creatin, could be held in solution by the water of a probable minimum amount of urine excreted in twenty-four hours.

A quarter of an hour before the time for beginning an experiment the bladder was emptied, and just before the experiment determinations were made of the pulse rate, of the rate of breathing, of the bodily temperature (taken under the tongue), and of the reaction of the urine, test paper being used. At a definite time, generally either 10 or 10.30 a. m., about two hours after breakfast, the dose of creatin or creatinin, previously reduced to fine powder, was swallowed with a little water.

With the larger doses there was perceived in a very short time—ten or fifteen minutes—a slight frontal headache, with a sense of constriction across the forehead, and slight ringing in the ears like that produced by quinin. These symptoms, which were of but trifling intensity, were accompanied by slight general nervous agitation, and did not last for more than an hour or two. For several hours there was a decided tendency to urinate frequently.

These general effects seemed to be the same for both the flesh bases but were rather more marked in the case of creatin than in that of creatinin. No disturbance of digestion was perceptible from any of the doses used.

The pulse and respiration rates, the bodily temperature, and the reaction of the urine to litmus paper were observed at definite intervals. Exactly twenty-four hours after the time at which a dose had been swallowed the bladder was again emptied; the whole of the urine

for the intervening day, collected in a single vessel, was measured, weighed, and the specific gravity noted after the liquid had cooled to 15° C. The portions submitted to analysis were weighed.

The following table shows the amount of creatinin or creatin taken, the amount of urine excreted, and its specific gravity in seven experiments:

Creatinin and creatin taken and urine excreted.

Number of experiment.	Creatinin taken.	Creatin taken.	Urine excreted in 24 hours.	Specific gravity of urine.
	Grams.	*Grams.*	*C. c.*	
1	5	.....	1,267	1.024
2	10	.....	1,415	1.026
3	15	.....	1,544	1.029
4	.....	3	1.502	1.023
5	.....	5	1,358	1.024
6	.....	10	1,529	1.027
7	.....	15	1,464	1.029

The pulse rate, rate of respiration, body temperature, and reaction of the urine in the different tests were as follows:

Physiological effect of creatinin and creatin.

	Pulse rate per minute.	Temperature.	Respiration per minute.	Reaction of urine.
Experiment No. 1:		*F.*		
Just before taking creatinin	81	98.5	18	Normally acid.*a*
One-half hour after taking creatinin	73	98.5	18	Do.
One hour after taking creatinin	69	98.6	17	Do.
Two hours after taking creatinin	70	98.5	18	Do.
Five hours after taking creatinin	79	98.6	19	Do.
Experiment No. 2:				
Just before taking creatinin	80	98.3	17	Decidedly acid.
One-half hour after taking creatinin	70	98.2	17	A shade less acid.
One hour after taking creatinin	*b* 62	97.9	15	Very faintly acid.
Two hours after taking creatinin	*c* 74	98.4	18	Distinctly acid, but not quite so much so as at beginning.
Five hours after taking creatinin	83	98.3	17	Acidity fully normal.
Experiment No. 3:				
Just before taking creatinin	76	98.6	19	Normally acid.
One-half hour after taking creatinin	66	98.3	17	Acidity a shade less than normal.
One hour after taking creatinin	*d* 57	97.7	16	Preceptibly acid, but less so than at beginning.
Two hours after taking creatinin	*e* 72	98.2	18	Acidity a shade less than normal.
Five hours after taking creatinin	80	98.4	17	Acidity fully normal.
Experiment No. 4:				
Just before taking creatin	77	98.6	16	Normally acid.
One-half hour after taking creatin	76	98.5	16	Do.
One hour after taking creatin	74	98.6	17	Do.
Two hours after taking creatin	77	98.6	18	Do.
Five hours after taking creatin	79	98.5	17	Do.
Experiment No. 5:				
Just before taking creatin	83	98.5	19	Do.
One-half hour after taking creatin	78	98.5	18	Do.
One hour after taking creatin	74	98.3	19	Distinctly less acid than at first.
Two hours after taking creatin	77	98.5	18	Nearly of normal acidity.
Five hours after taking creatin	82	98.4	18	Normally acid.

a The exact degree of acidity was not measured by means of a standard alkaline solution, but estimated by the rapidity of development and the intensity of color produced on litmus paper of uniform character.

b With frequent and well-marked intermittence.

c With occasional slight intermittence.

d With marked intermittence.

e Intermittence scarcely perceptible.

Physiological effect of creatinin and creatin—Continued.

	Pulso rate per minute.	Temperature.	Respiration per minute.	Reaction of urine.
Experiment No. 6:		° F.		
Just before taking creatin	79	98.5	17	Rather more than normally acid.
One-half hour after taking creatin	73	98.4	17	Faintly acid.
One hour after taking creatin	69	98.5	16	Slightly alkaline.
Two hours after taking creatin	69	98.1	18	Very slightly alkaline.
Five hours after taking creatin	81	98.5	16	Acidity about normal.
Experiment No. 7:				
Just before taking creatin	78	98.4	17	Acidity fully normal.
One-half hour after taking creatin	70	98.4	18	Acidity a little less than normal.
One hour after taking creatin	67	98.3	18	Distinctly, but not strongly, alkaline.
Two hours after taking creatin	65	98.2	16	Very faintly alkaline.
Five hours after taking creatin	85	98.3	18	Practically neutral; slightly inclining to acid.

As will be seen, the most decided physiological effect produced was the retardation of the action of the heart. This was unmistakable. It seemed to be accompanied by little, if any, change in the force of impulse as felt in the radial artery; if there was any effect of this kind, it probably was a diminution of force, but there was no clear impression of such a change. The statements of others as to the flesh bases acting most perceptibly as nerve *stimulants* may perhaps be partly accounted for by smaller doses having been used by the author, and partly by evidence having been drawn from the use of meat extracts and the action of other constituents of these extracts having been confounded with the action of the pure flesh bases. The effect of creatinin as a cardiac retarder seems to be greater than that of creatin for the same dose. The latter seems also to act more slowly, this fact suggesting that its effect may be exerted, either wholly or chiefly, after it has undergone conversion into creatinin in the body, though this is hardly probable.

In the figures obtained there may possibly be traced a slight tendency to retardation of breathing and to lowering of bodily temperature, but there is no clear evidence of this.

An interesting point is the reduction of the acidity of the urine by creatin, extending, even in the case of the larger doses, to development of an alkaline reaction. Something of the same sort is observable for the larger doses of creatinin, though not to the same extent. On noticing the results of the tests made at different times after the ingestion of the flesh bases, it will be seen that the effect upon acidity, as well as that upon the heart's action, seems to have been most marked about an hour after the doses had been taken, with some tendency to longer delay in the case of creatin than in that of creatinin. In all the experiments the reaction of the mixed total excretion for twenty-four hours was acid, and apparently of about the normal intensity.

Applying to the seven specimens of urine of these experiments the method of analysis which has been described, the following results were obtained:

Constituents of urine excreted in twenty-four hours when flesh bases were taken.

Number of experiment.	Urea.	Creatinin.	Creatin.
	Grams.	*Grams.*	*Gram.*
1	29.096	5.386	.....
2	29.457	10.097	.....
3	29.558	14.816	.....
4	28.973	3.170	.....
5	28.735	4.742	0.014
6	30.011	8.741	.069
7	29.714	12.699	.177

Examining these results, there seems to be a small increase of urea—an average of 29.363 grams per day as against the average of 28.922 grams obtained from the nine samples of normal urine—and perhaps a little tendency to higher figures in the experiments in which the larger doses of the flesh bases were taken. But neither of these conclusions can be drawn with any certainty, since differences between the individual results, both in the case of normal urine and also when flesh bases were taken, were greater than the difference between the two averages.

The figures for creatinin include, of course, the amount of this substance *normally* present in the urine, and therefore the amount found in each experiment must be reduced by 0.817 gram, the average value for normal creatinin as found in the nine experiments first recorded. Assuming also, on the basis of the same nine experiments, that the analytical process employed yields 95 per cent of the quantity of creatinin really present, the following are the corrected results for experiments 1, 2, and 3, when creatinin was taken:

Corrected results of tests in which creatinin was taken.

Number of experiment.	Creatinin ingested.	Creatinin recovered.	Creatinin recovered as percentage of creatinin ingested.
	Grams.	*Grams.*	*Per cent.*
1	5	4.809	96.18
2	10	9.768	97.68
3	15	14.736	98.24

As regards creatin, it is evident at a glance that nearly all of that swallowed was converted into creatinin and eliminated in this latter form, very small amounts, however, escaping and undergoing elimination unchanged. This fact of any creatin escaping change in the rapid passage through the system of a large dose is in itself an interesting point, and such an apparent result is not probably to be explained by the supposition of change in the opposite direction having occurred during the process of analysis. It is to be noticed that the quantities of creatin found as such—in all cases small—increase with increase of the dose swallowed. In order to see how far creatin was recovered, the quantity of creatinin found has first to be reduced by the allowance for that normally present, then increased in the ratio of

95:100, so as to correspond with the determined degree of accuracy of the analytical method used, and then calculated to the equivalent quantity of creatin which it represents—the quantity of creatin found as such must be increased in the ratio of 86.9:100, so as to correspond with the determined degree of accuracy of the analytical method—and finally the creatin thus found really present as such must be added to that represented by creatinin. The corrected results for experiments 4, 5, 6, and 7 are as follows:

Corrected results of tests in which creatin was taken.

Number of experiment.	Creatin ingested.	Creatin recovered as such or represented by creatinin.	Creatin recovered as percentage of creatin ingested.
	Grams.	*Grams.*	*Per cent.*
4	3	2.872	95.73
5	5	4.806	96.12
6	10	9.749	97.49
7	15	14.703	98.02

Looking at the actual quantities of creatinin and creatin recovered, it is seen that the loss on the quantity ingested does not vary largely in the several experiments, being only somewhat greater for the larger doses than the smaller; hence the relative percentage loss is less for the former than for the latter. For both urea and creatinin it has only been possible to allow for the quantity present in the urine in its natural condition on the basis of the average of the determinations made; it must be remembered that this, therefore, conceals such differences as might have appeared if the exact normal condition of the excretion could have been ascertained on the particular day on which creatin or creatinin was administered. It is, moreover, conceivable that the normal excretion of urea or creatinin, or both, may have been altered by the very ingestion of either of the flesh bases, though this is hardly likely.

CONCLUSIONS.

The main conclusion to be drawn is manifestly this: that by far the larger part of the flesh bases ingested, if not absolutely the whole, does not undergo metabolism with the production of urea or anything else, but on the contrary is eliminated by way of the kidneys. In the case of creatinin it is excreted unchanged, while creatin is changed wholly or very largely into creatinin.

This elimination in the urine of the flesh bases is reflected in the figures giving the specific gravity of the excretion. Two experiments only were made, with a view to ascertaining whether any of the creatin or creatinin taken in heavy doses could be recovered from the aqueous extract of the feces, it seeming possible that the comparatively slight solubility of creatin in particular might lead to some of it escaping absorption along the course of the intestinal canal. In these two cases the results were altogether negative.

The fact of the quantitative recovery of creatin and creatinin from the urine evidently accords fully with the generally accepted belief that these substances can not serve to build up proteids, and therefore are not to be classed among tissue-forming food materials.

On the whole, this investigation is unfavorable to the idea of the creatin of living musele being the main antecedent of urea in nitrogenous metabolism. It is of course conceivable that the creatin of the living museles may be *slowly* metabolized with production of urea, and that, as Voit has remarked, the cause of the ingested flesh bases appearing in large amount in the urine, instead of undergoing conversion into urea, may be that time enough has not been allowed for metabolism, but this is rendered very improbable by the large quantity of urea given off daily. It can hardly be admitted that such slow and difficult formation of urea from creatin can possibly represent the regular course of change by which the bulk of the urea is produced. It must be remembered, of course, that creatin swallowed, as in the above experiments and getting into the blood by absorption from the alimentary canal, does not follow exactly the same course and is not placed under exactly the same conditions as that found in and derived from the living muscles. However, it is not easy to imagine such a difference in the fate of the same substance from these two sources, once having become a constituent of the blood, as would account for its being in the one case metabolized and in the other eliminated unchanged or merely converted into its anhydrid, creatinin.

However this may be, and admitting that it is still an unsolved problem what nitrogenous substance or substances may properly be regarded as intermediate between muscle proteids and urea, it may fairly be regarded as established for nutrition investigations that the so-called flesh bases, creatin and creatinin, occurring in food may be entirely disregarded as sources of energy, being excreted practically without having undergone change.

This conclusion seems to be deserving of attention on the one hand by the analyst of food materials and on the other by the physician who prescribes and the consumer who makes use of the ordinary meat extracts.

In the discussion of the results of analyses of meat and forms of food prepared from it, such as soups and the like, it is evidently wrong and misleading to confound together, under the head of protein or proteid materials, the proteids proper, capable of building up the nitrogenous tissues of the living body and of furnishing muscular energy and heat by oxidation, and these so-called flesh bases, which, taken in along with food, are not available for either of these important purposes. This error is the more serious that creatin and creatinin, containing so large a percentage of nitrogen as they do, appear to represent and are counted as representing much more than their own weight of nutrient material, whereas they should be excluded altogether in food analyses from the nutrient material really present.

As regards the practical use of meat extracts, those forms of such preparations from which the proteids and peptones have been removed may well be considered as destitute of nutritive value, and those in which, along with some proteid material, the flesh bases occur in large quantity may be considered as deriving no nutritive value from this latter source.

Even if viewed in the light of nerve stimulants only, and thus to be classed with tea and coffee as adjuncts to food rather than as true food itself, meat extracts, so far as the flesh bases, creatin and creatinin, are concerned, are shown by this investigation to be very much less active in their effects upon the nervous system than they have been commonly reputed. The potassium salts present in large quantity in meat extracts are by no means to be overlooked as influencing such effects as are produced upon the living organism, and it may be that some of the minor organic constituents are relatively more potent than creatin and creatinin, but aside from such real proteid or peptonic material as is in some cases present, the meat extract preparations may fairly be relegated on the whole to the class of condiments or flavoring matters rather than to that of foods or medicines.

SUGGESTIONS FOR FURTHER INVESTIGATIONS.

It is desirable that the following points, suggested by this investigation, should, if possible, be examined. To this end it might be desirable—

(1) By experiments, with other persons as subjects, to ascertain the influence, if any, of age, sex, etc., upon the behavior of the flesh bases in the living organism.

(2) To try the effect of a given amount of creatin or creatinin, administered at once as a single dose, as compared with the same quantity divided into several parts, administered as separate doses and distributed over several hours with but short intervals.

(3) To try to recover creatin or creatinin (administered by the mouth) from the *blood* of one of the lower animals killed or heavily bled soon after receiving a large dose of the flesh base. This would involve the interesting question whether creatinin can undergo conversion into creatin in the alkaline blood and such reaction be afterwards reversed in connection with elimination by the kidneys.

(4) To examine, not the whole urinary excretion of twenty-four hours, but several samples of urine taken at short successive intervals for two or three hours after the ingestion, by swallowing, of a large dose of creatin or creatinin, so as to get a more definite idea of the rapidity with which elimination takes place, to obtain further evidence as to creatin being capable of passing through the renal filter without conversion into creatinin, and to see whether the reverse change can occur.

(5) To examine more fully the observed reversal of the normal acid reaction of the urine after ingestion of a large amount of creatin.

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